1.3 [illegible]

1.目的基因的获取

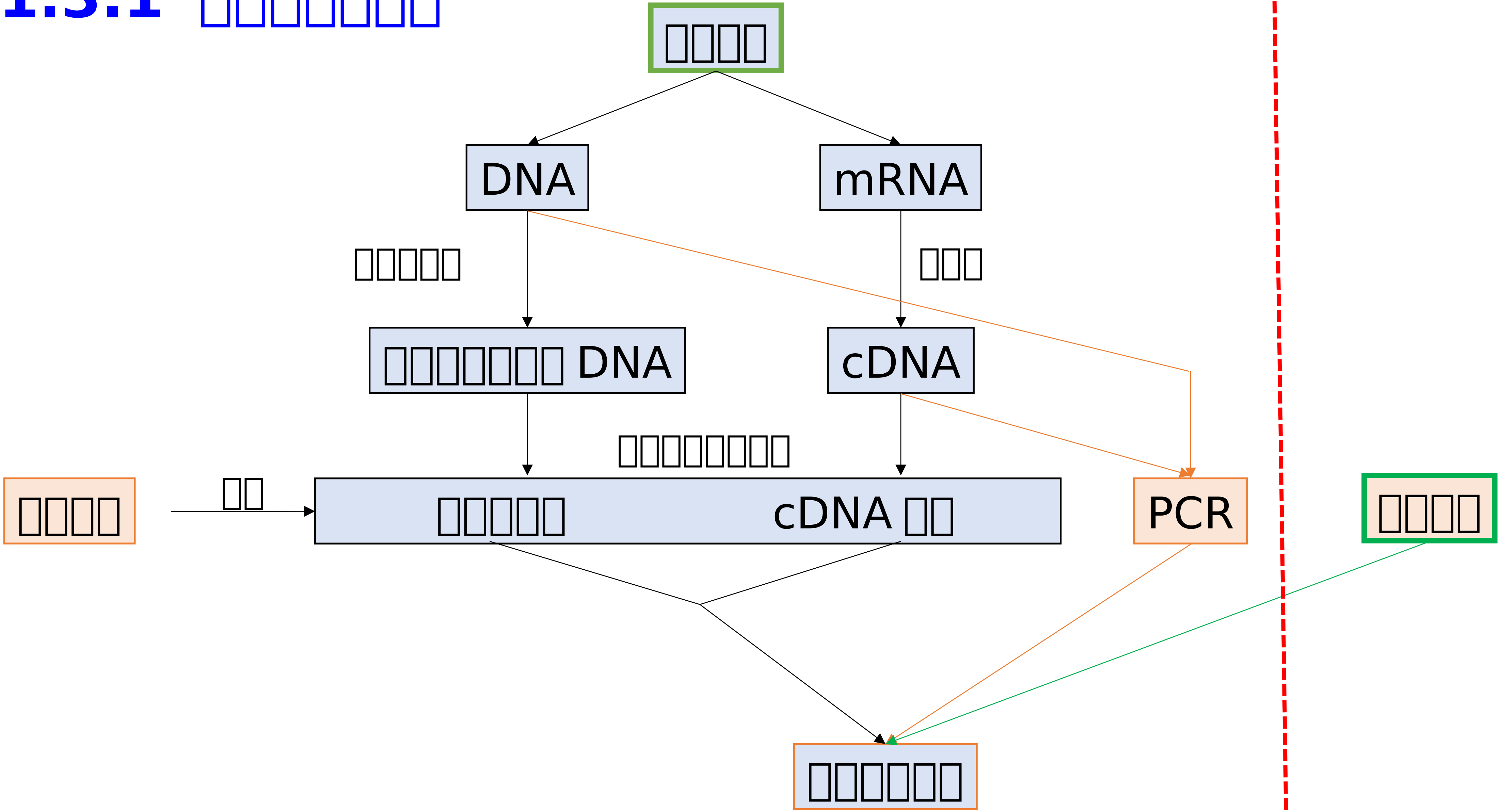
4.目的基因的检测与鉴定

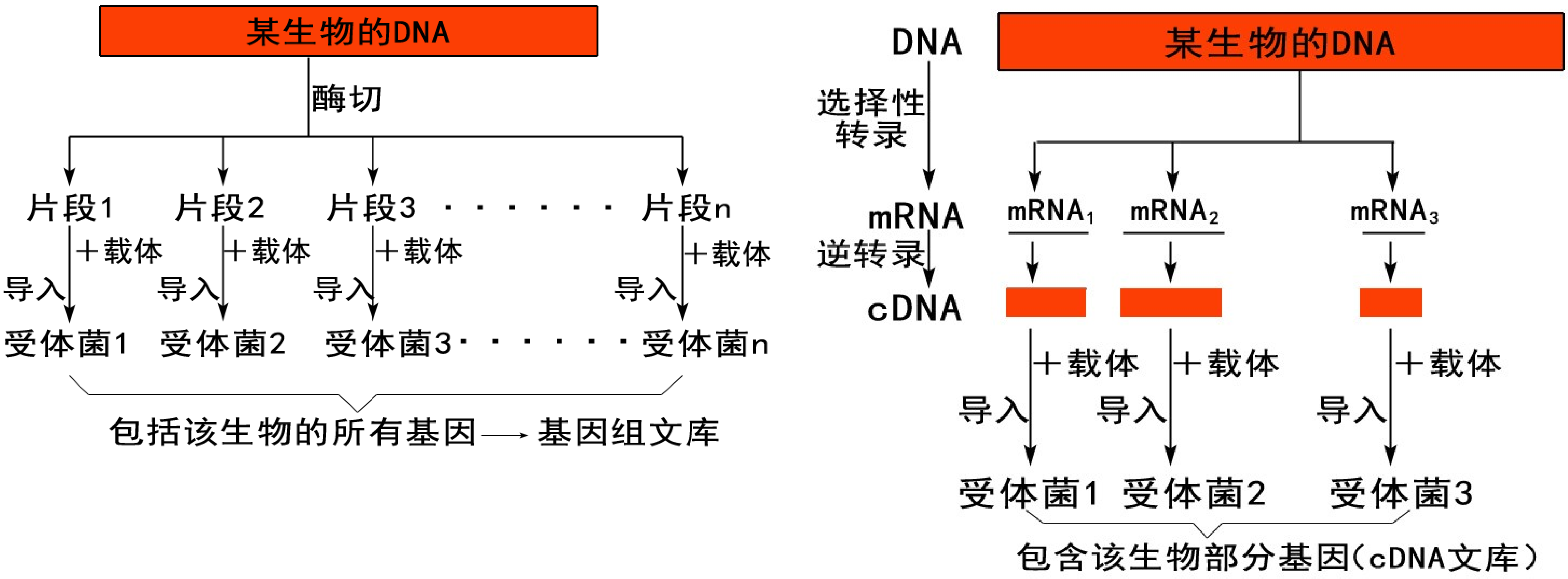
基因工程的基本操作程序

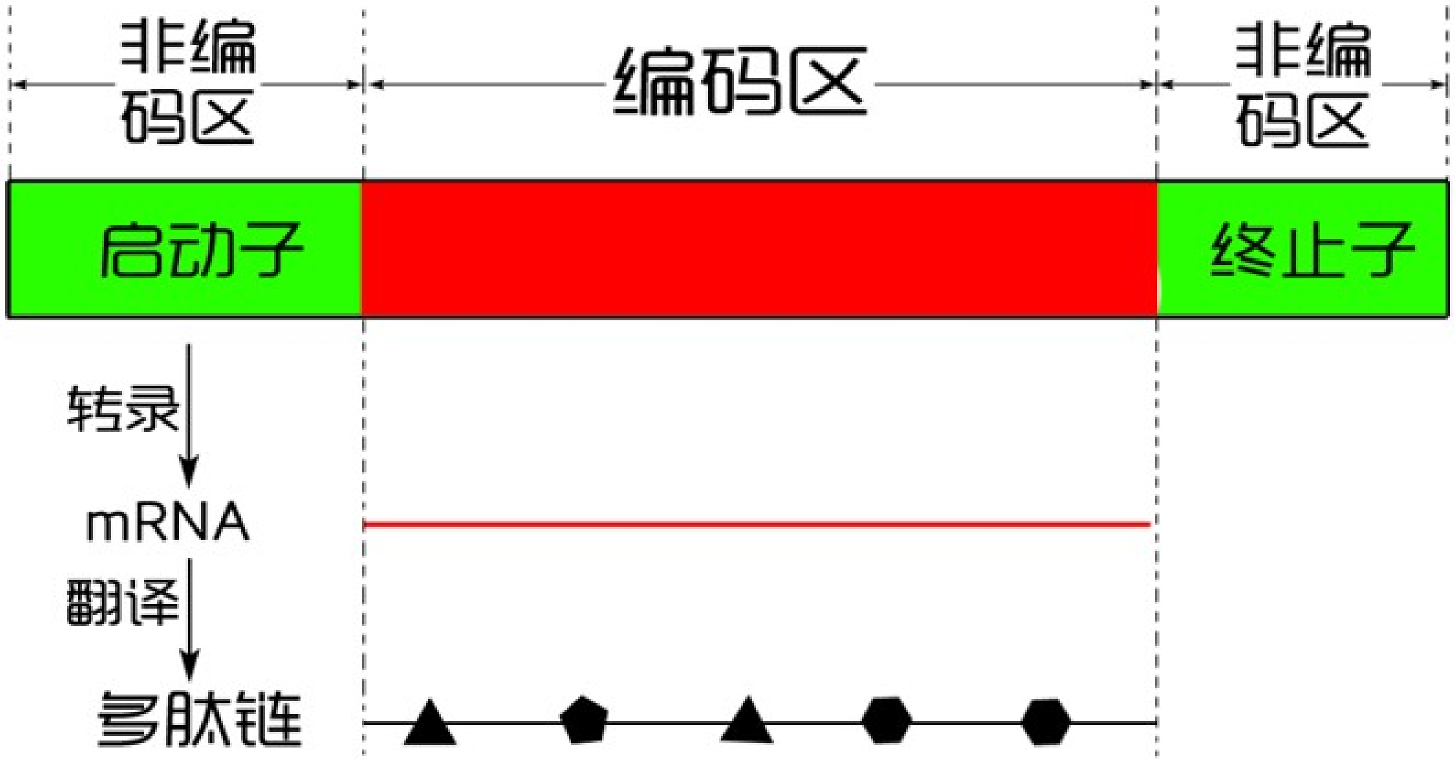
2.基因表达载体的构建(核心步骤)

3.将目的基因导入受体细胞

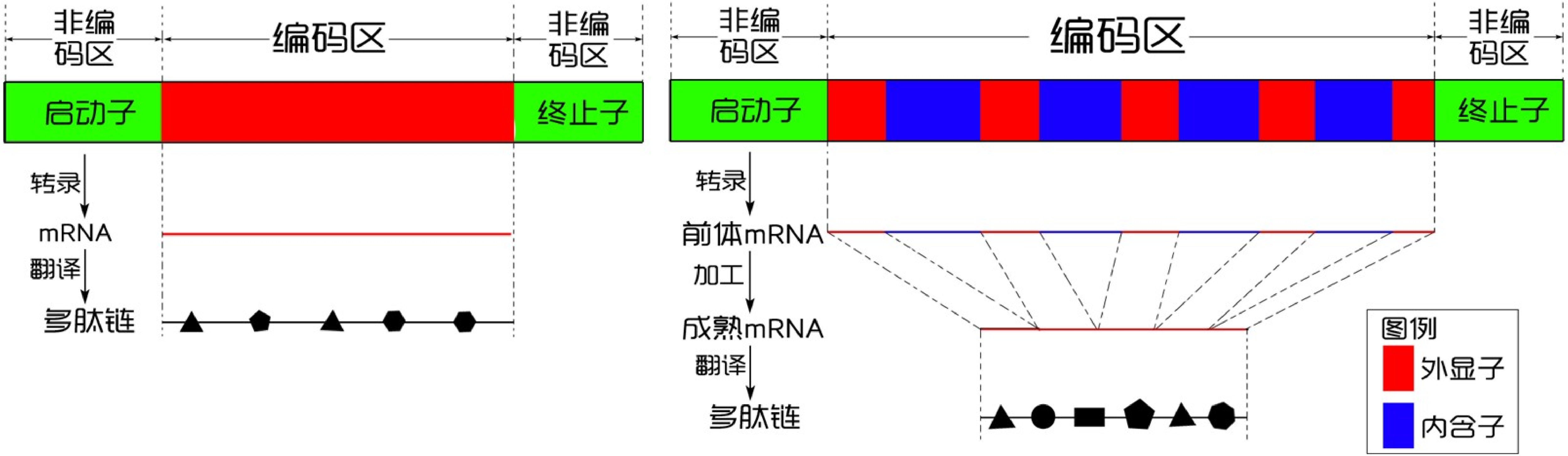
1.3.1




某生物的DNA
酶切
片段1
片段2
片段3
片段n
+载体
导入
受体菌1
受体菌2
受体菌3
受体菌n
包括该生物的所有基因→基因组文库
DNA
选择性转录
mRNA
逆转录
cDNA
某生物的DNA
mRNA1
mRNA2
mRNA3
+载体
导入
受体菌1
受体菌2
受体菌3
包含该生物部分基因(cDNA文库)



非编码区
编码区
非编码区
启动子
终止子
转录
mRNA
翻译
多肽链



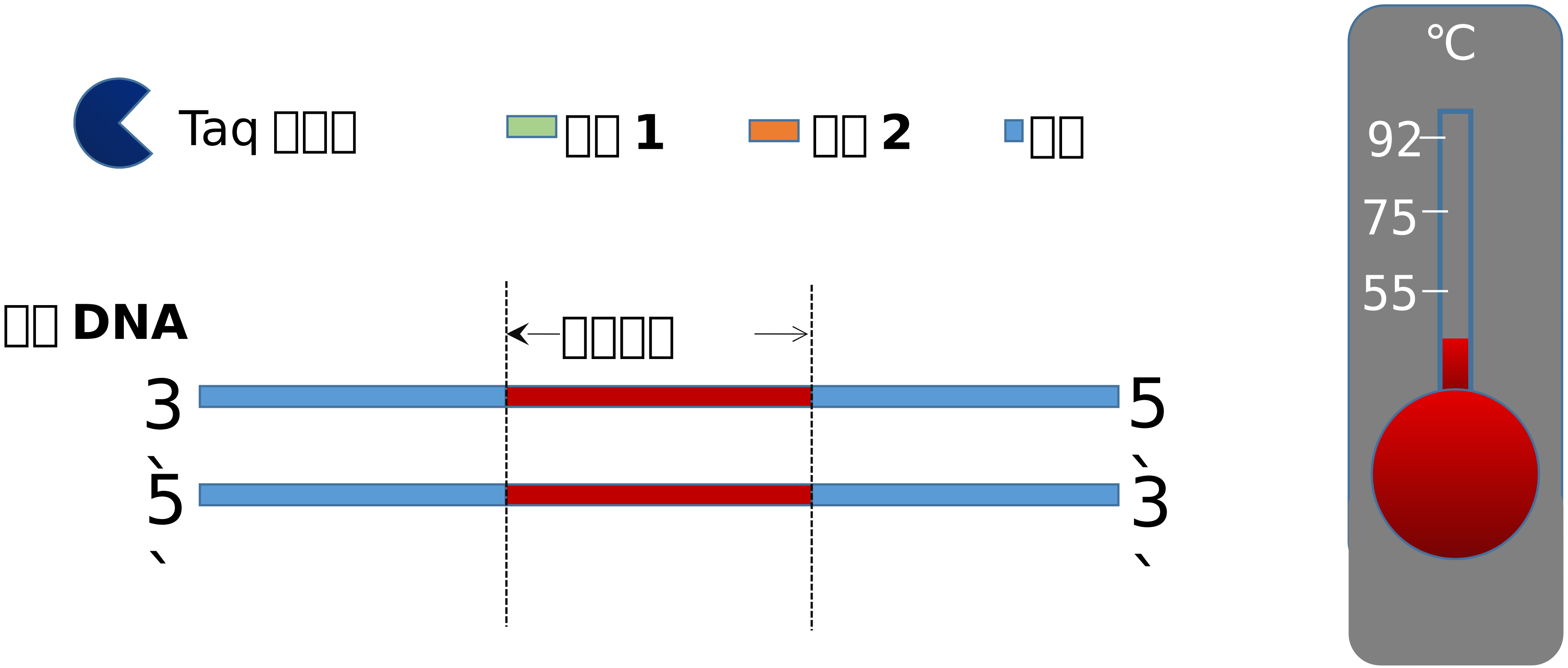
非编码区
编码区
非编码区
启动子
终止子
转录
mRNA
翻译
多肽链
非编码区
编码区
非编码区
启动子
终止子
转录
前体mRNA
加工
成熟mRNA
翻译
多肽链
图例
外显子
内含子

[illegible]	cDNA [illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]

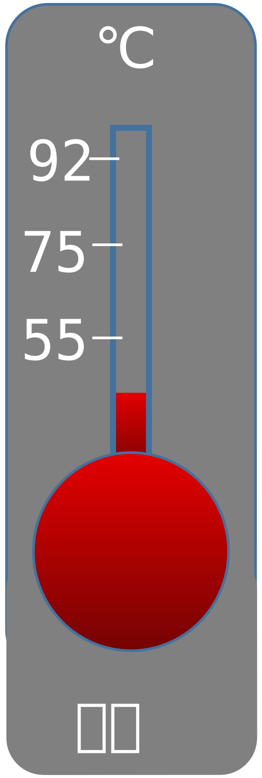
cDNA [illegible]

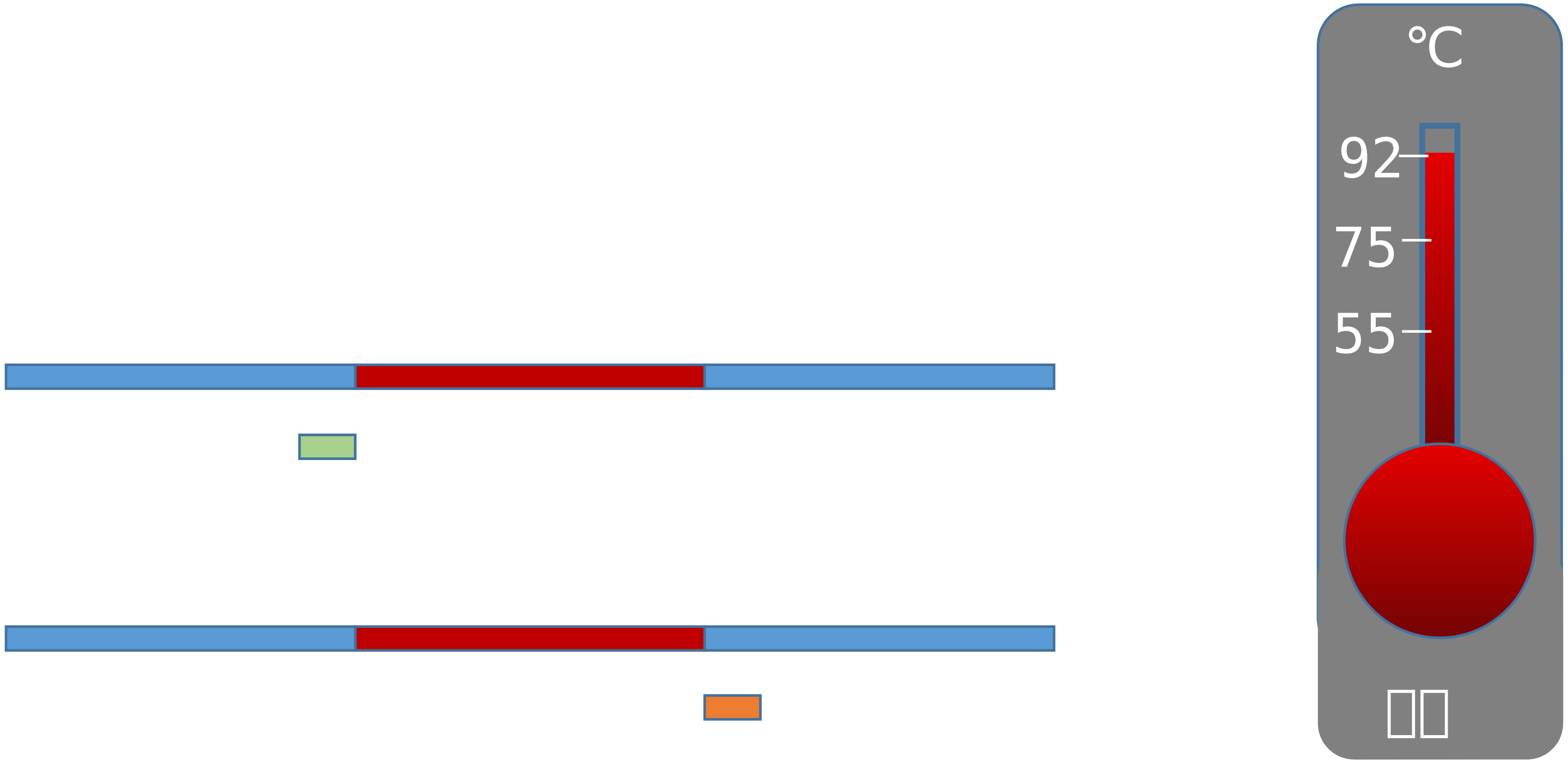
[illegible]

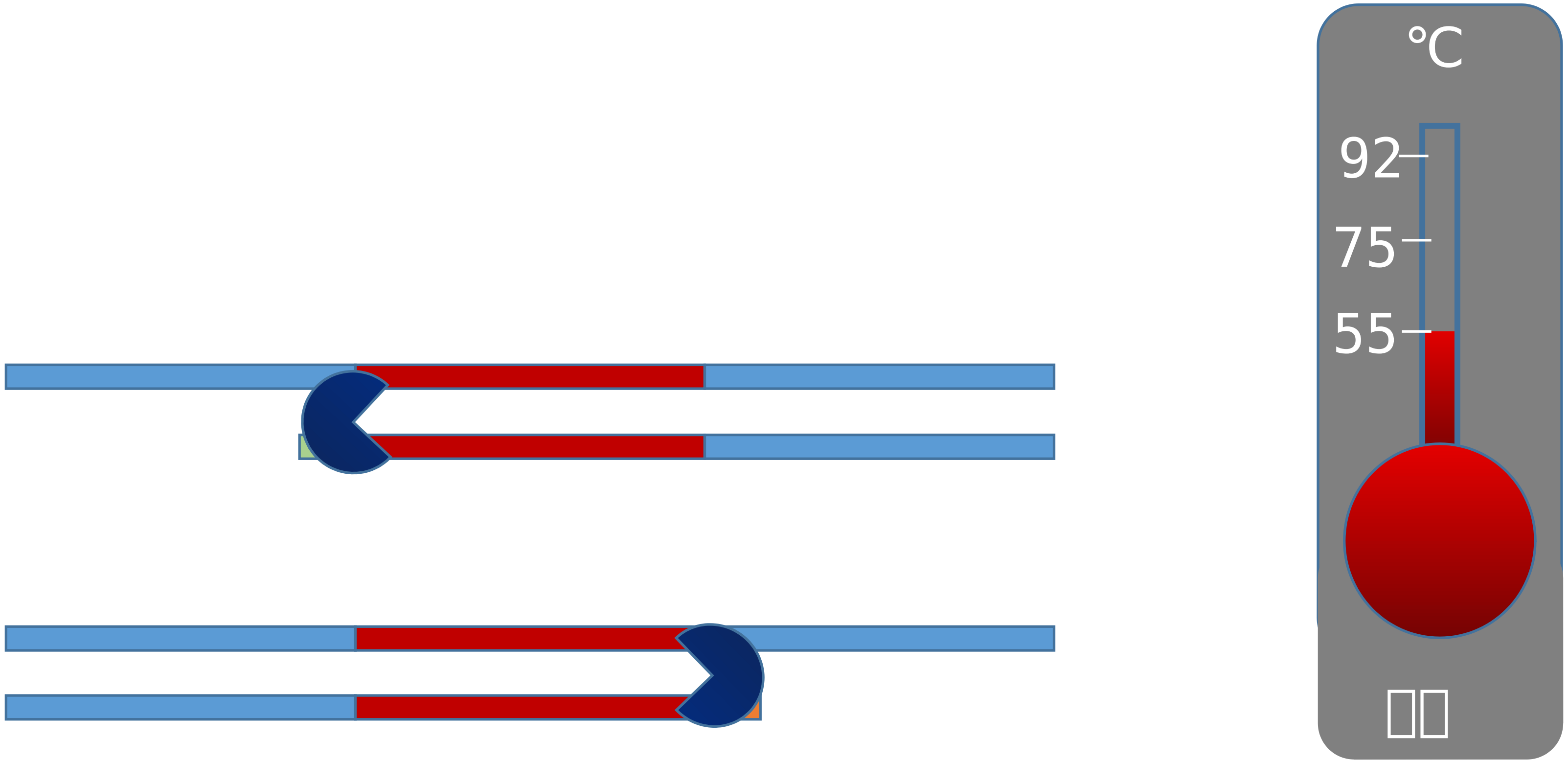
PCR [illegible]

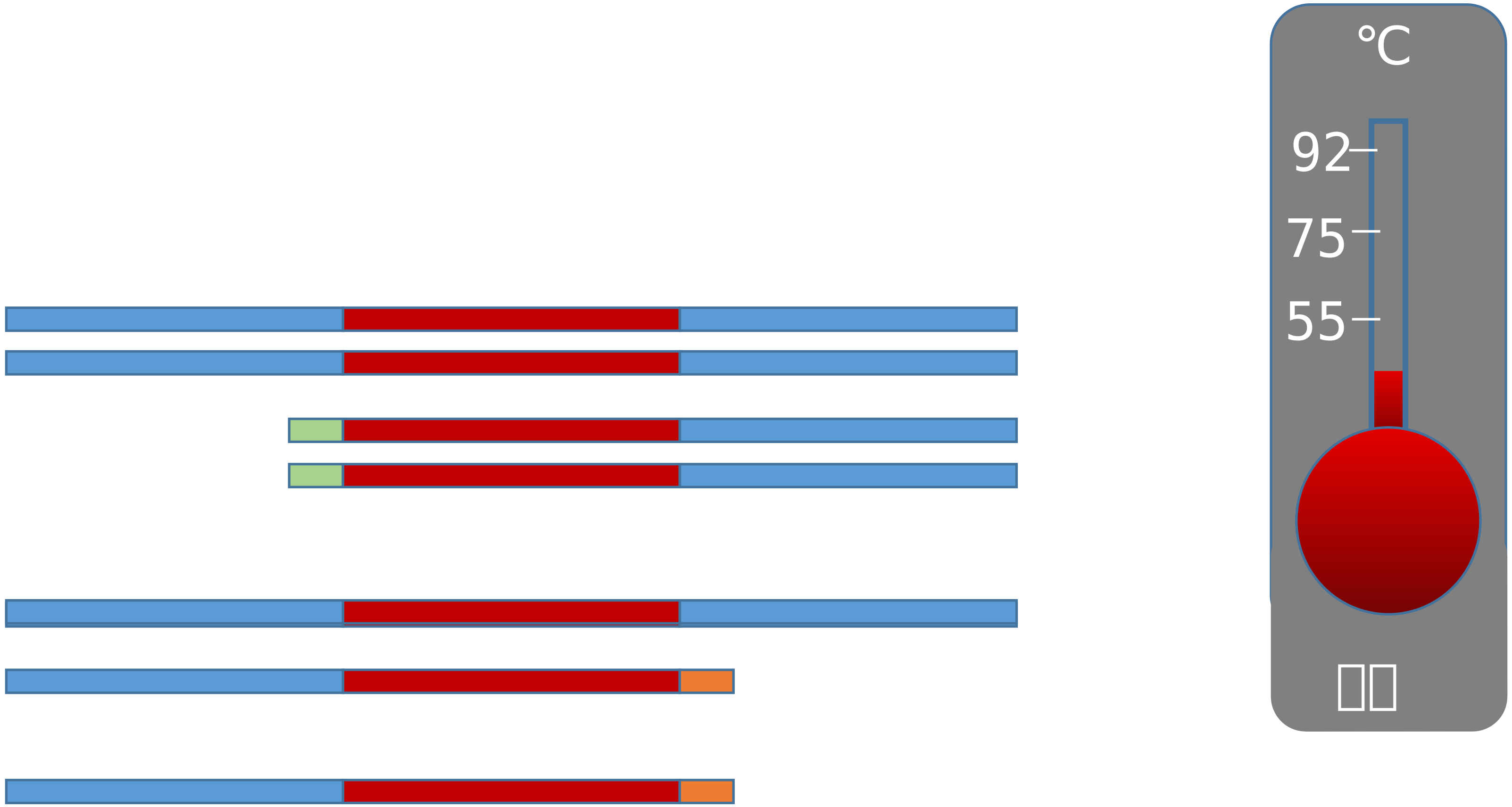


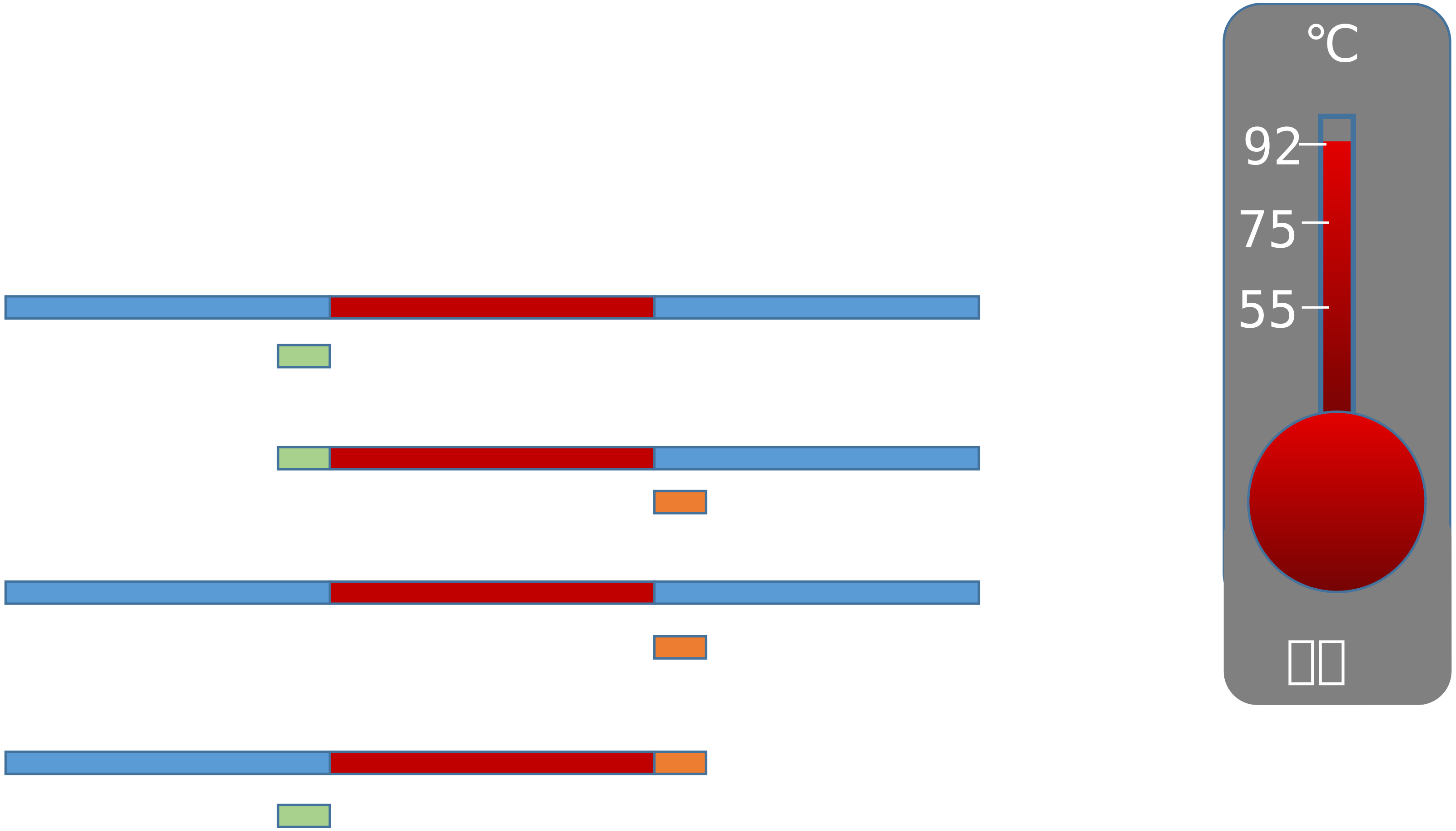
3`
5`
5`
3`

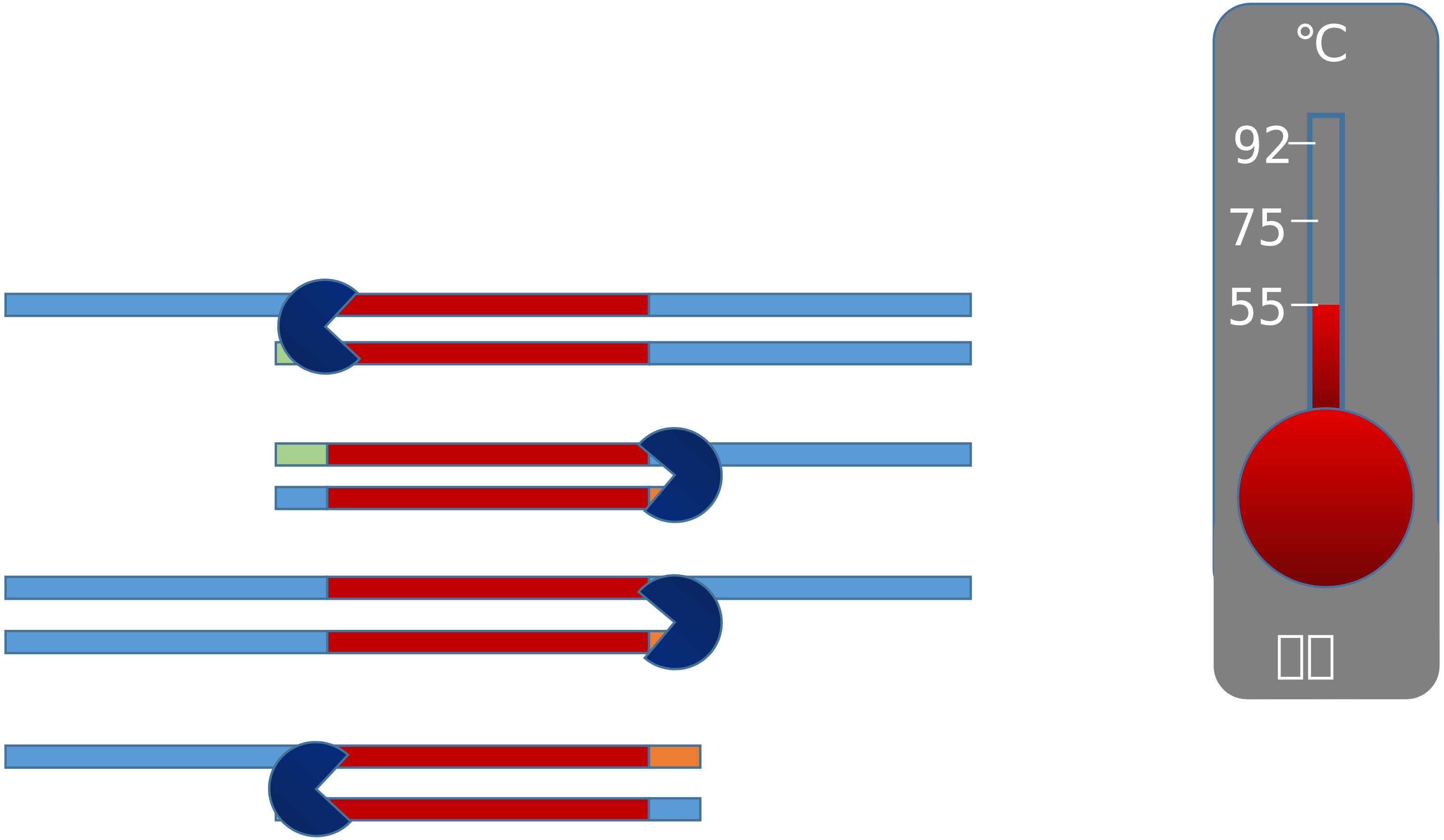

℃
92
75
55

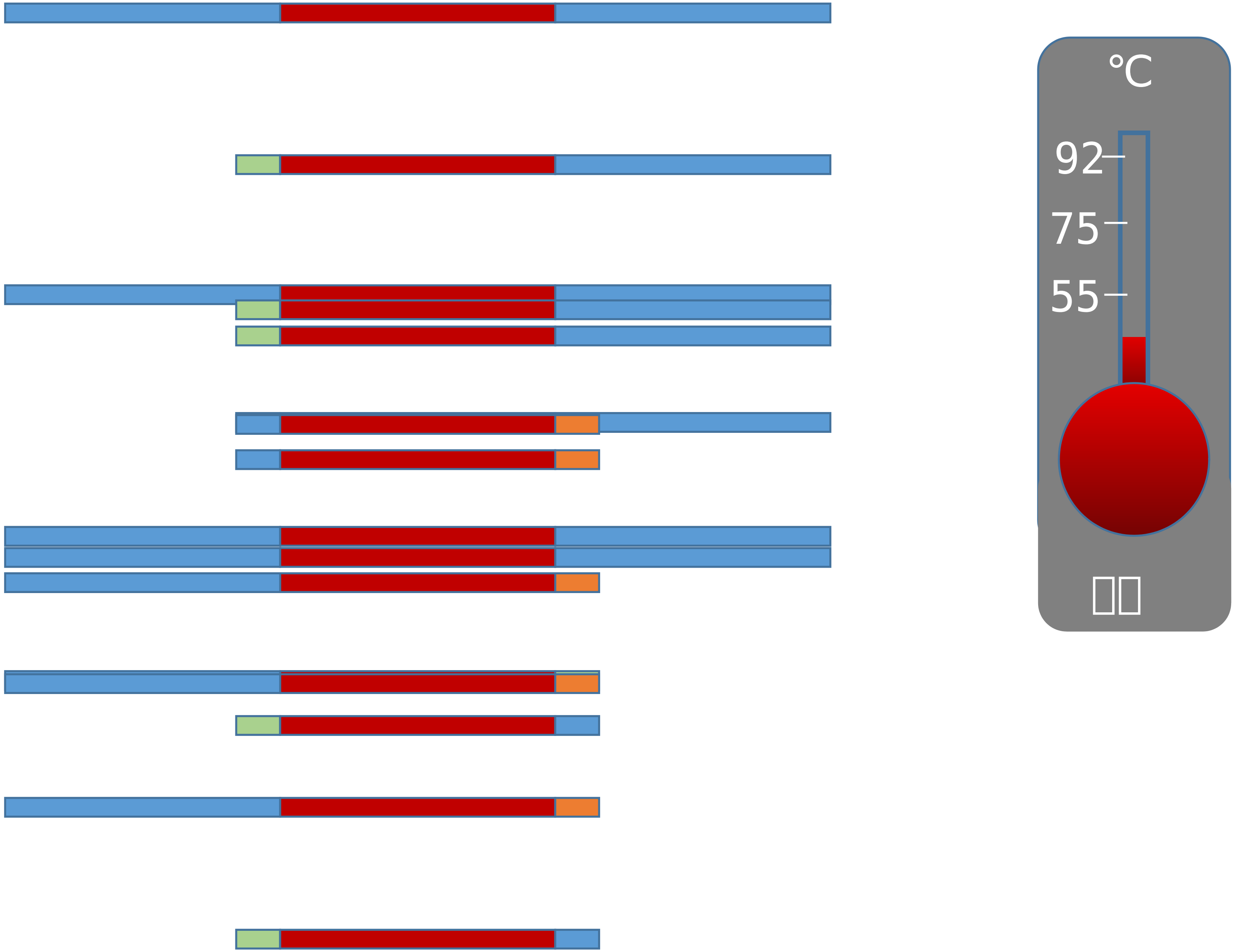

°C
92
75
55

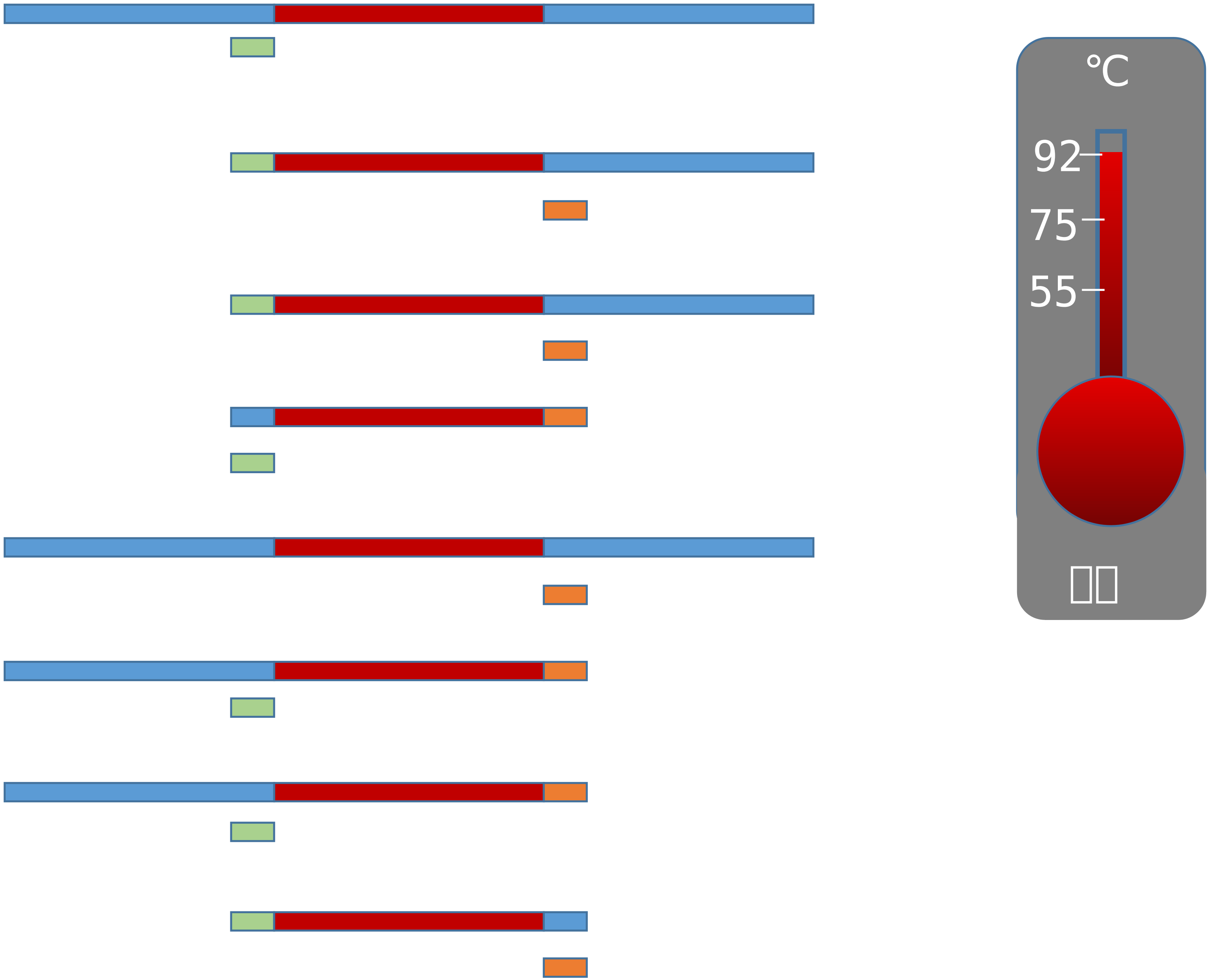

°C
92
75
55

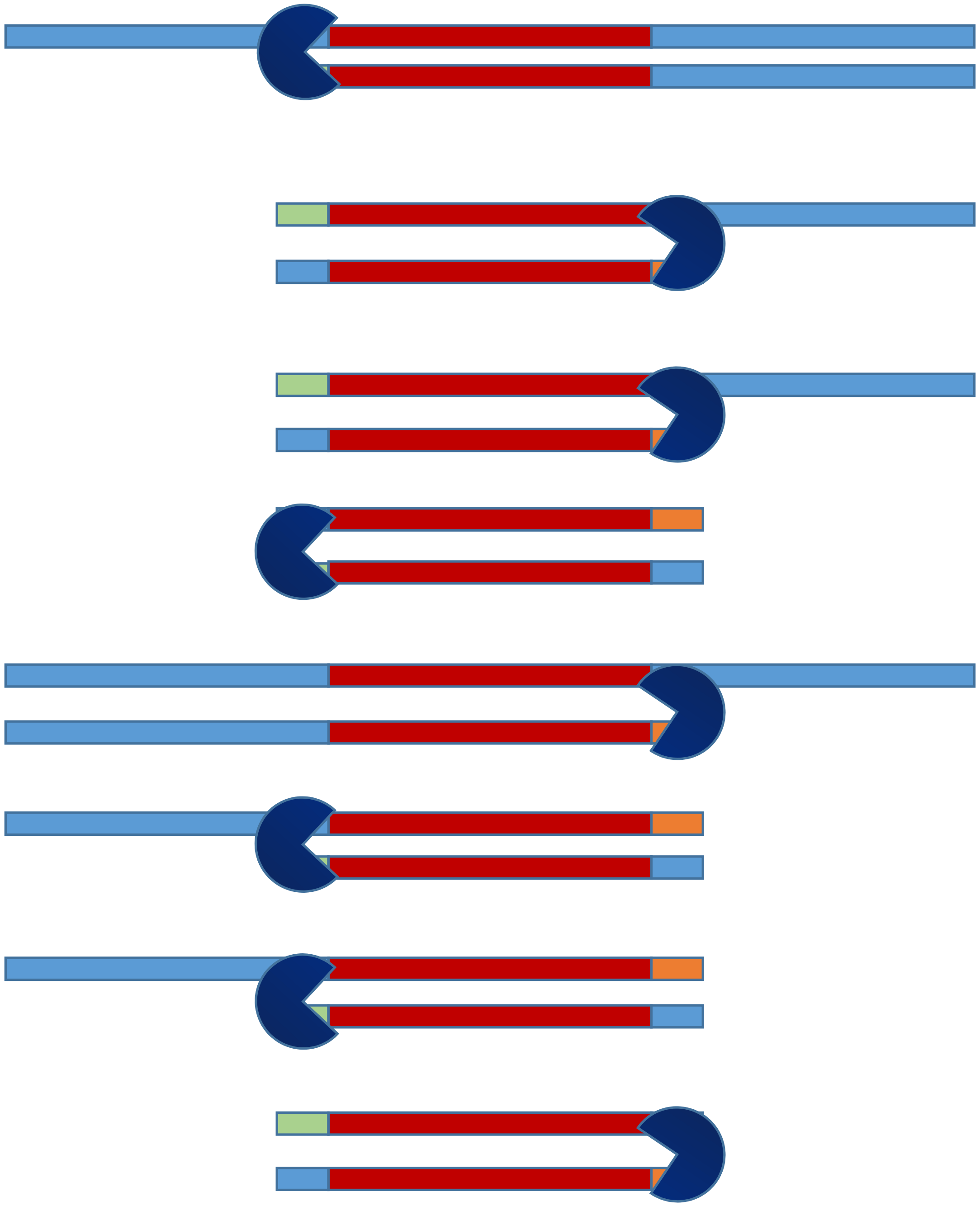

°C
92
75
55

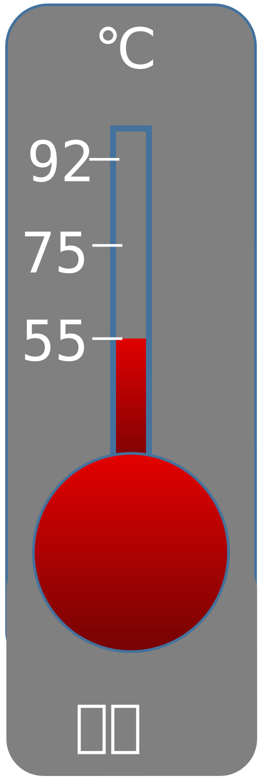

°C
92
75
55


°C
92
75
55


°C
92
75
55


°C
92
75
55




°C
92
75
55

PCR [illegible] [illegible] ______ [illegible] DNA [illegible] ______ [illegible]

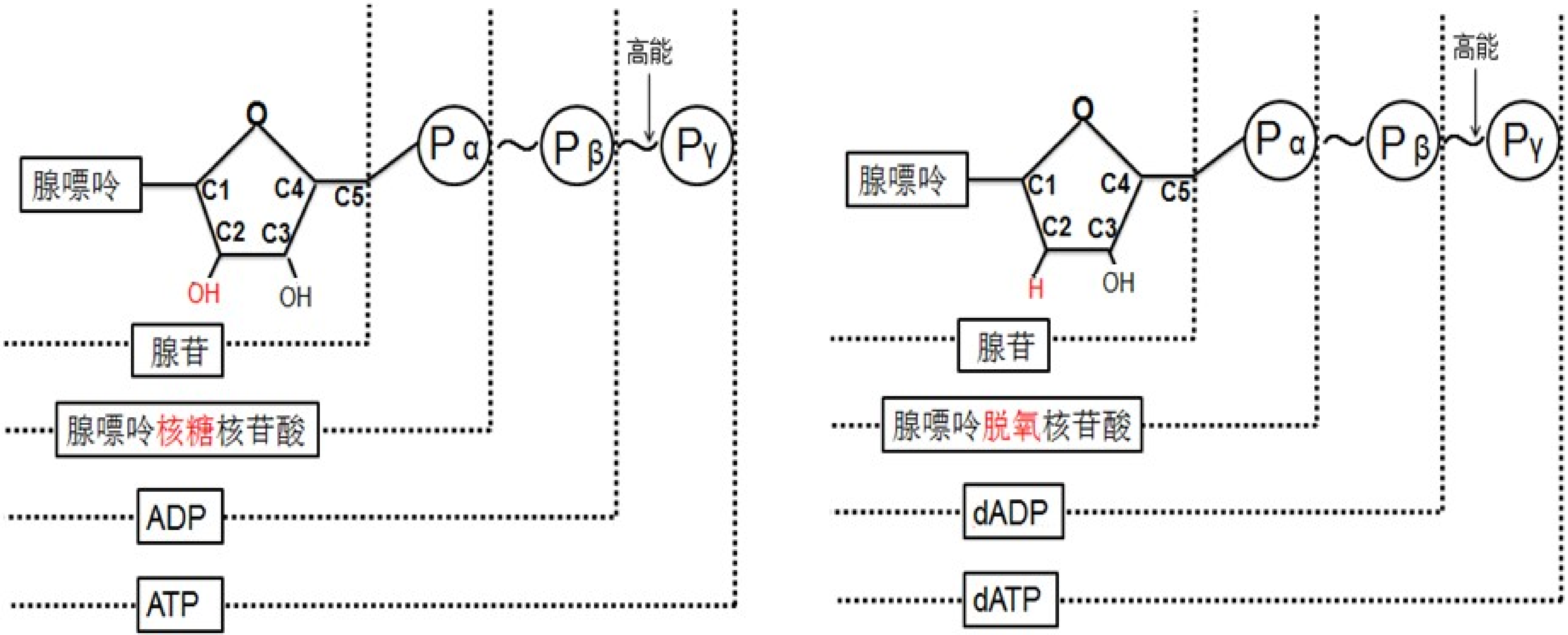
4 [illegible] ______ [illegible] dATP [illegible] dTTP [illegible] dGTP [illegible] [illegible] dCTP [illegible] [illegible] DNA [illegible] ______ [illegible]

______ [illegible] DNA [illegible] PCR [illegible]

[illegible] ______ [illegible] [illegible] ______ [illegible] [illegible] ______ [illegible] [illegible] 95℃ ______ [illegible] 55℃ ______ [illegible] 72℃ ______ [illegible]

[illegible] PCR [illegible] ______ [illegible] [illegible]“[illegible]”[illegible]“[illegible]”[illegible]“[illegible]”[illegible]

[illegible]

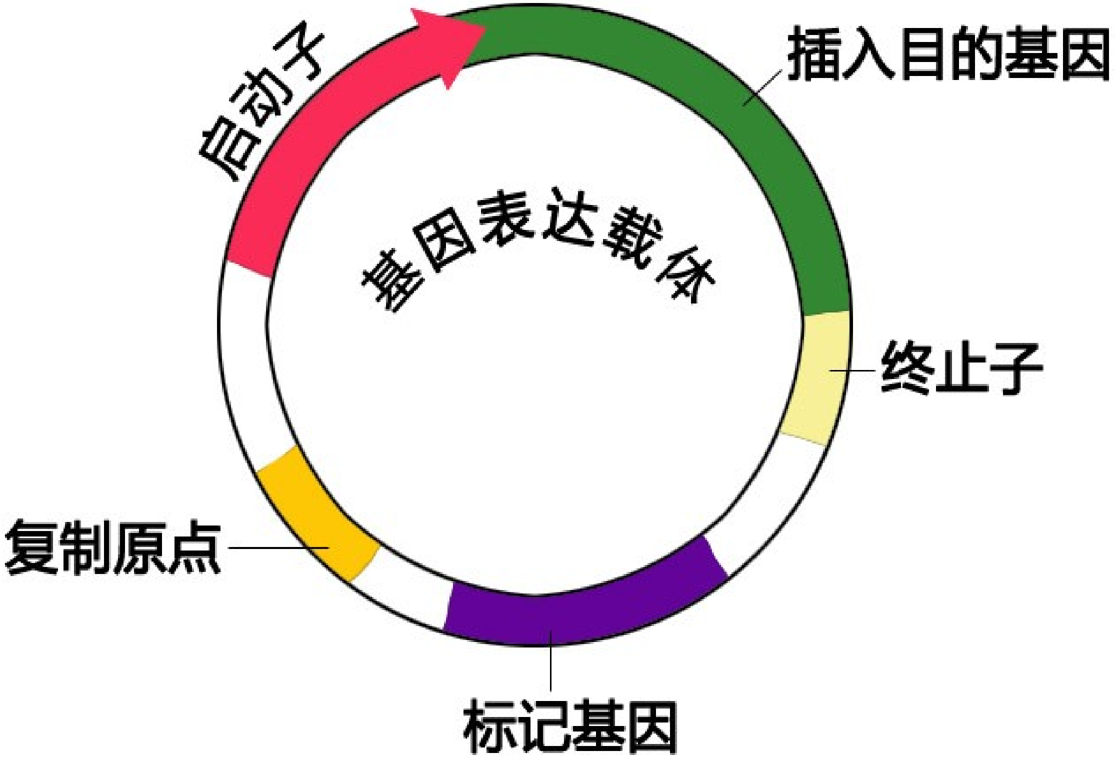

腺嘌呤
O
C1
C4
C5
C2
C3
OH
OH
Pα
Pβ
Pγ
高能
腺苷
腺嘌呤核糖核苷酸
ADP
ATP
腺嘌呤
O
C1
C4
C5
C2
C3
H
OH
Pα
Pβ
Pγ
高能
腺苷
腺嘌呤脱氧核苷酸
dADP
dATP

dNTP [illegible] PCR [illegible]，[illegible]

PCR [illegible] DNA [illegible]

[illegible]		[illegible] DNA [illegible]	PCR [illegible]
[illegible]	[illegible]	[redacted]	[redacted]
	[illegible]	[redacted]	[redacted]
	[illegible]	[redacted]	[redacted]
	[illegible]	[redacted]	[redacted]
	[illegible]	[redacted]	[redacted]
[illegible]		① [illegible] [redacted]	

1.3.2 [illegible]



[illegible]

[illegible]

[illegible]?

[illegible]

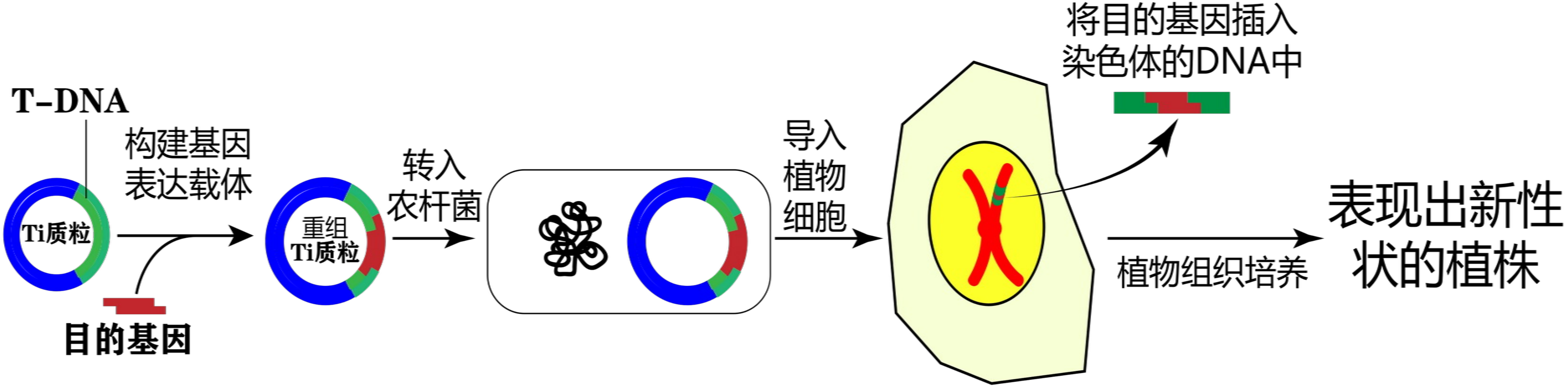
1.3.3 [illegible]

[illegible]

[illegible]?

[illegible]

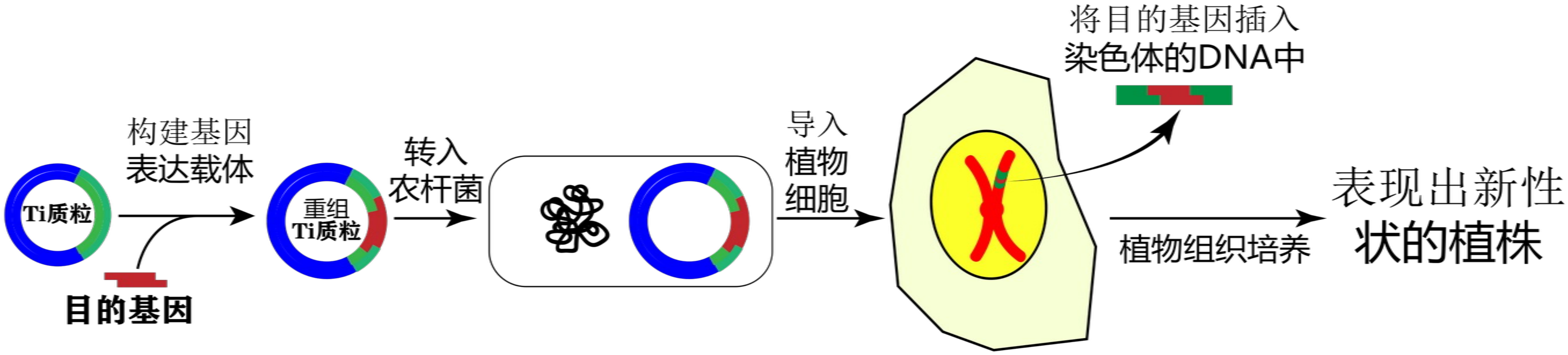
[illegible] ------ [illegible]



T-DNA [illegible] T [illegible] T-DNA [illegible]

[illegible] Ti [illegible]

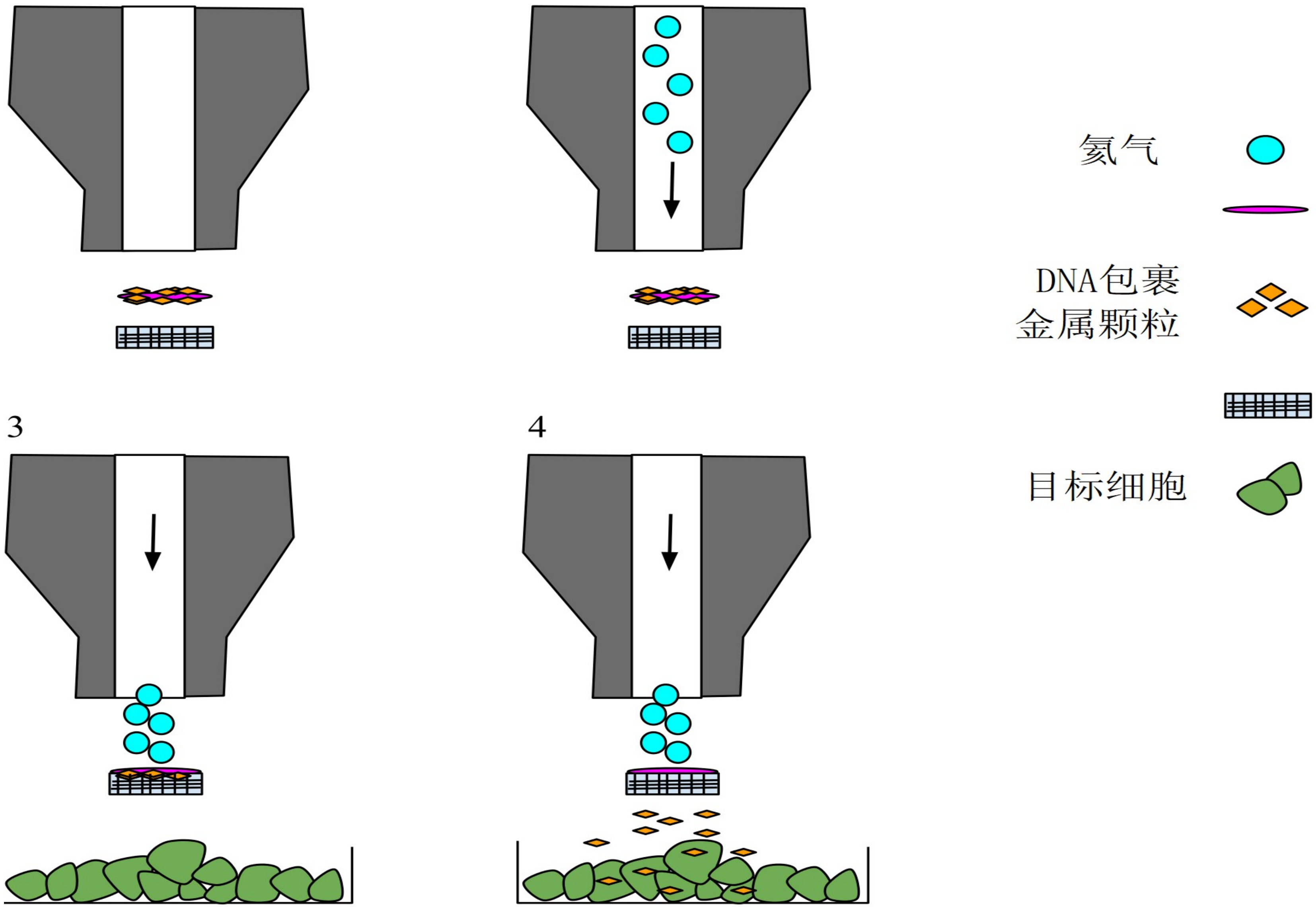
[illegible]



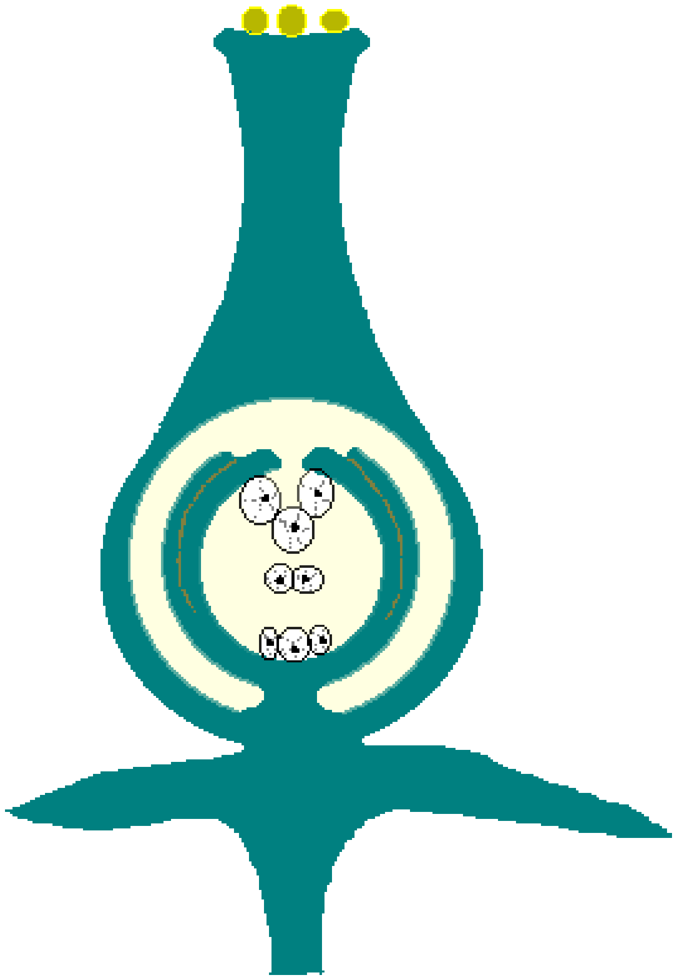
[illegible] Ti [illegible] T-DNA [illegible] ([illegible]) [illegible]
[illegible] T-DNA [illegible] DNA [illegible]

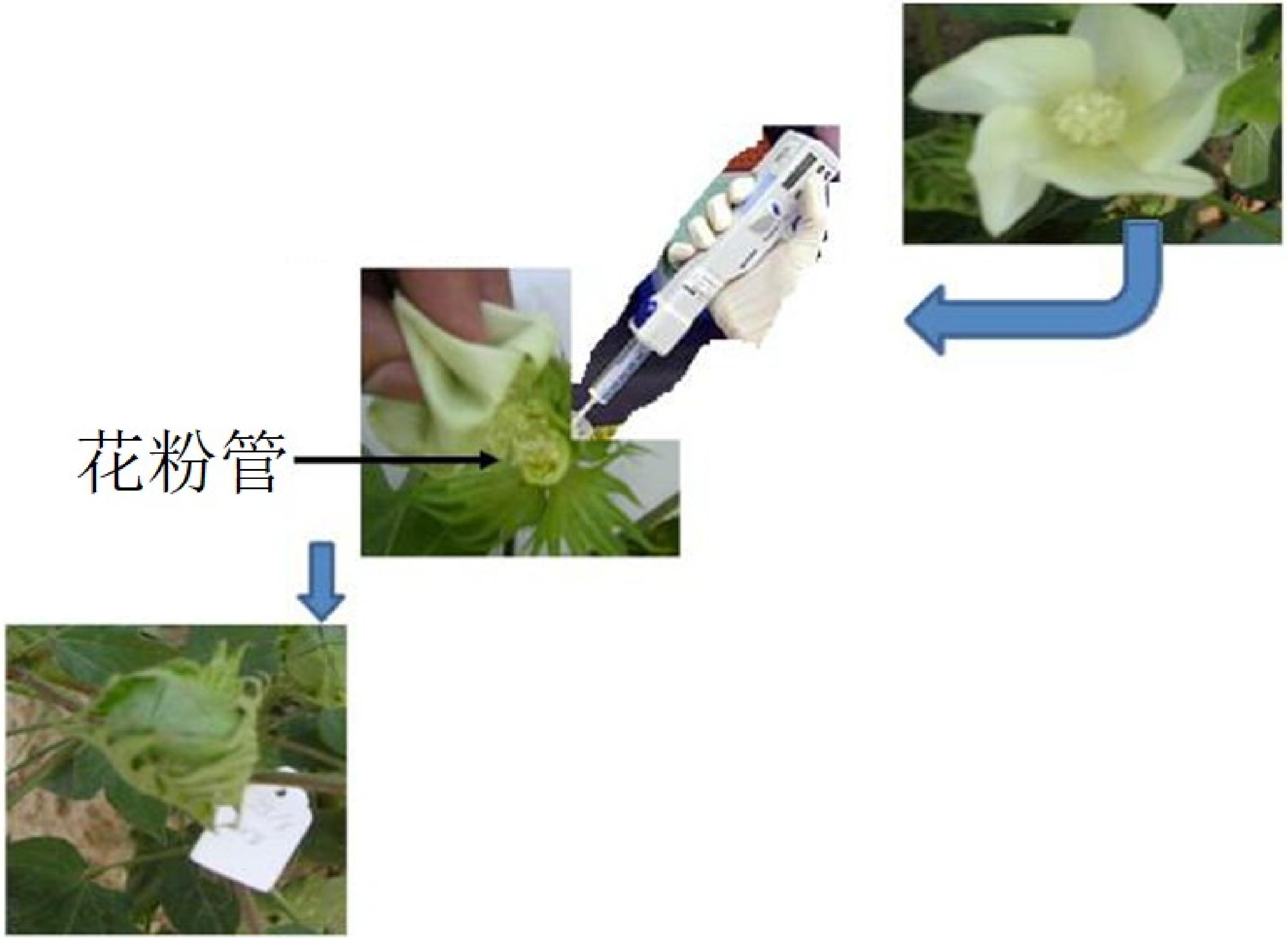
[illegible] Ti [illegible] ([illegible]) [illegible]
[illegible] T-DNA [illegible]

□□□□

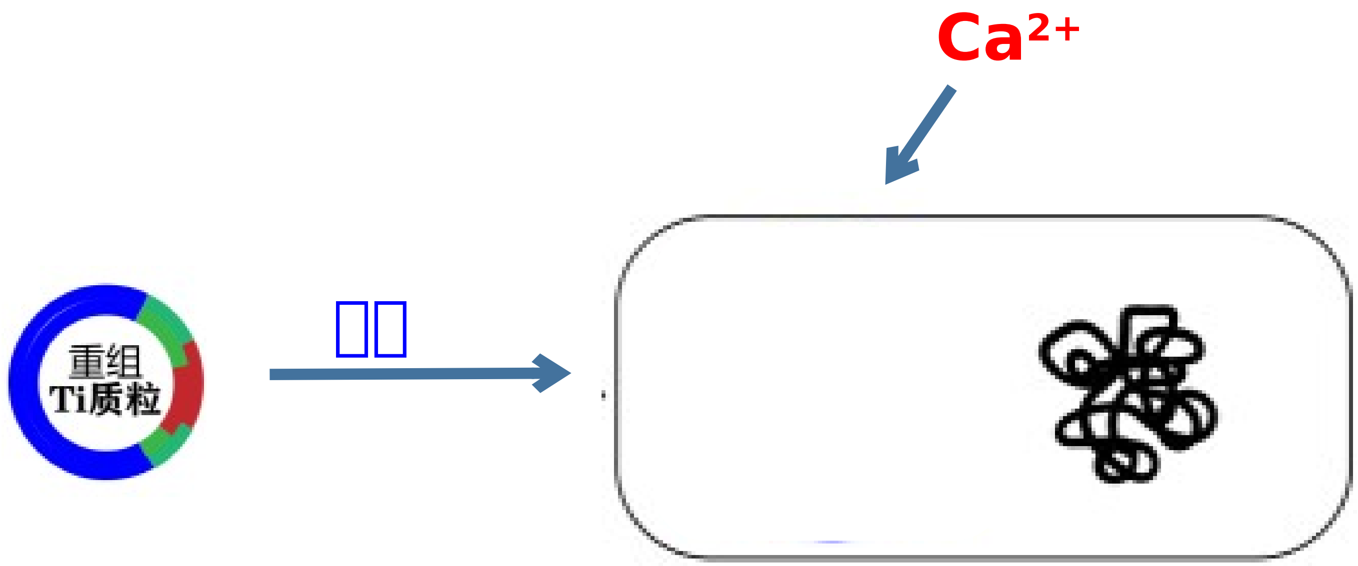

3
4
氦气
DNA包裹
金属颗粒
目标细胞

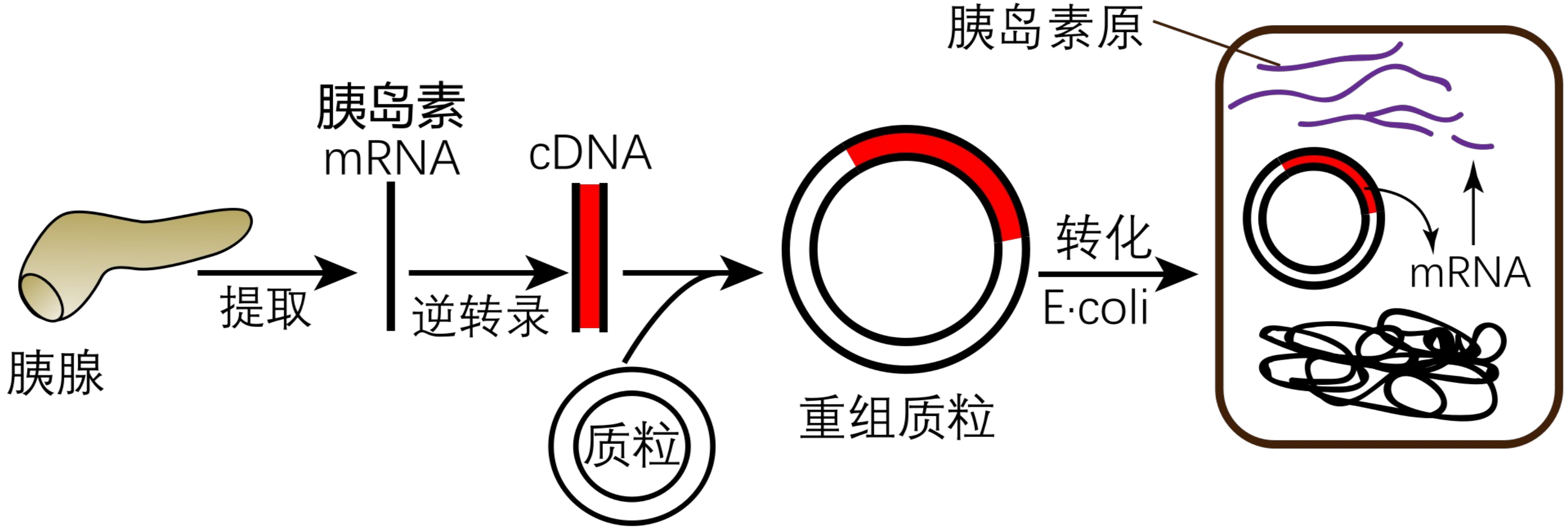
[illegible]

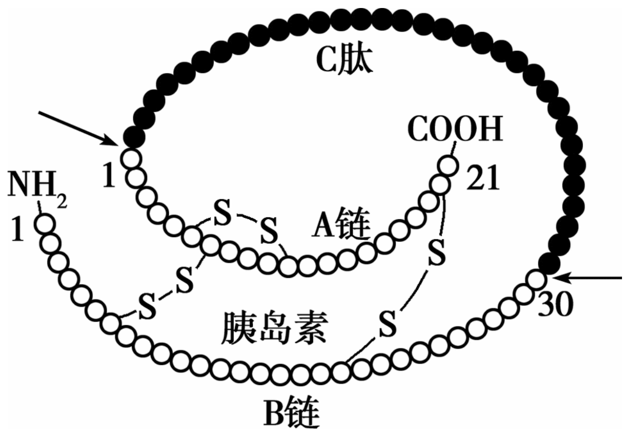



花粉管

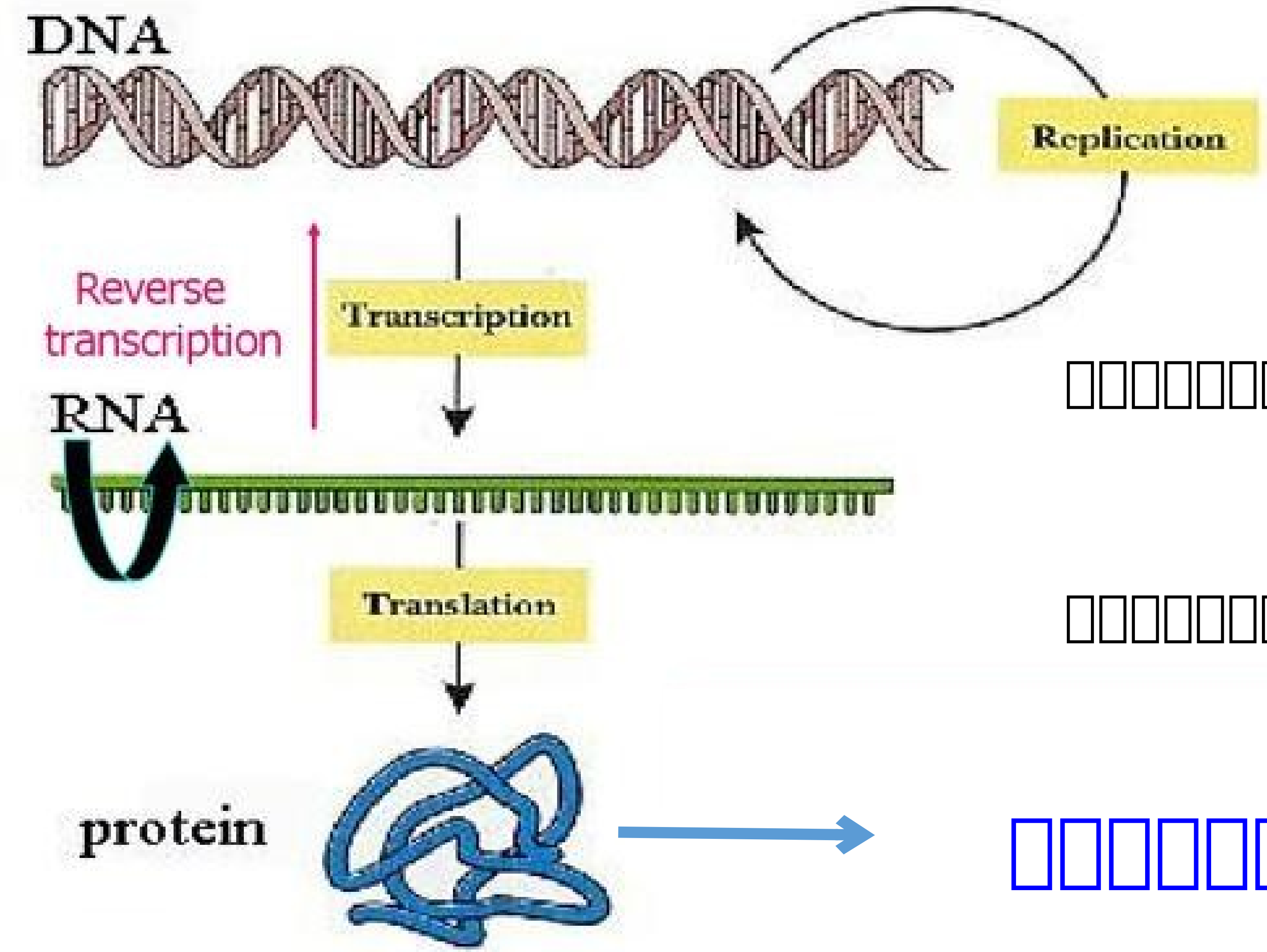
显微操作仪


Ca2+
重组
Ti质粒


胰腺
提取
胰岛素
mRNA
逆转录
cDNA
质粒
重组质粒
转化
E·coli
胰岛素原
mRNA


C肽
COOH
NH2
1
1
21
S
S
A链
S
S
S
S
S
S
30
胰岛素
B链

1.3.4 [illegible]



DNA
Replication
Reverse transcription
Transcription
RNA
Translation
protein

[illegible]

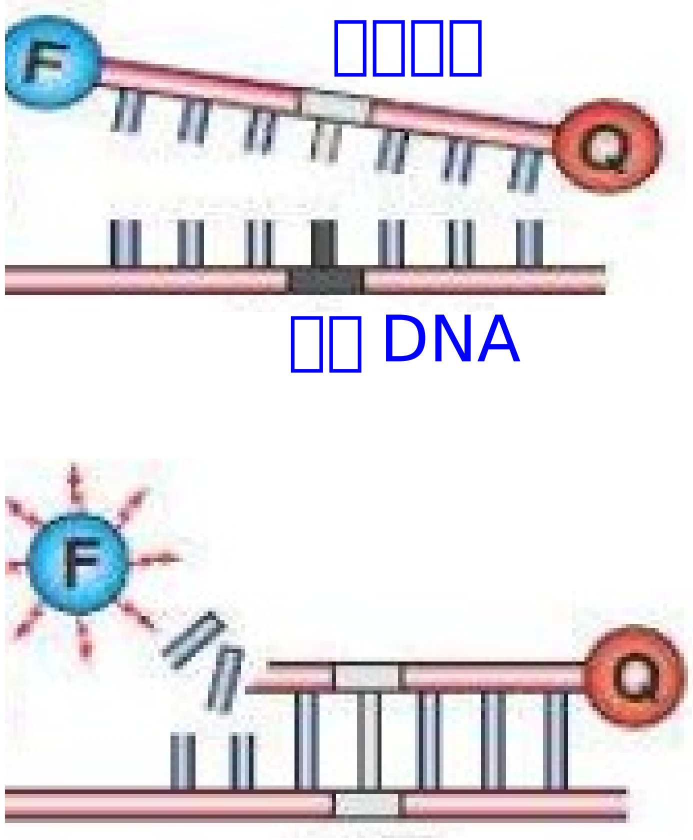
---- [illegible]

[illegible], [illegible]

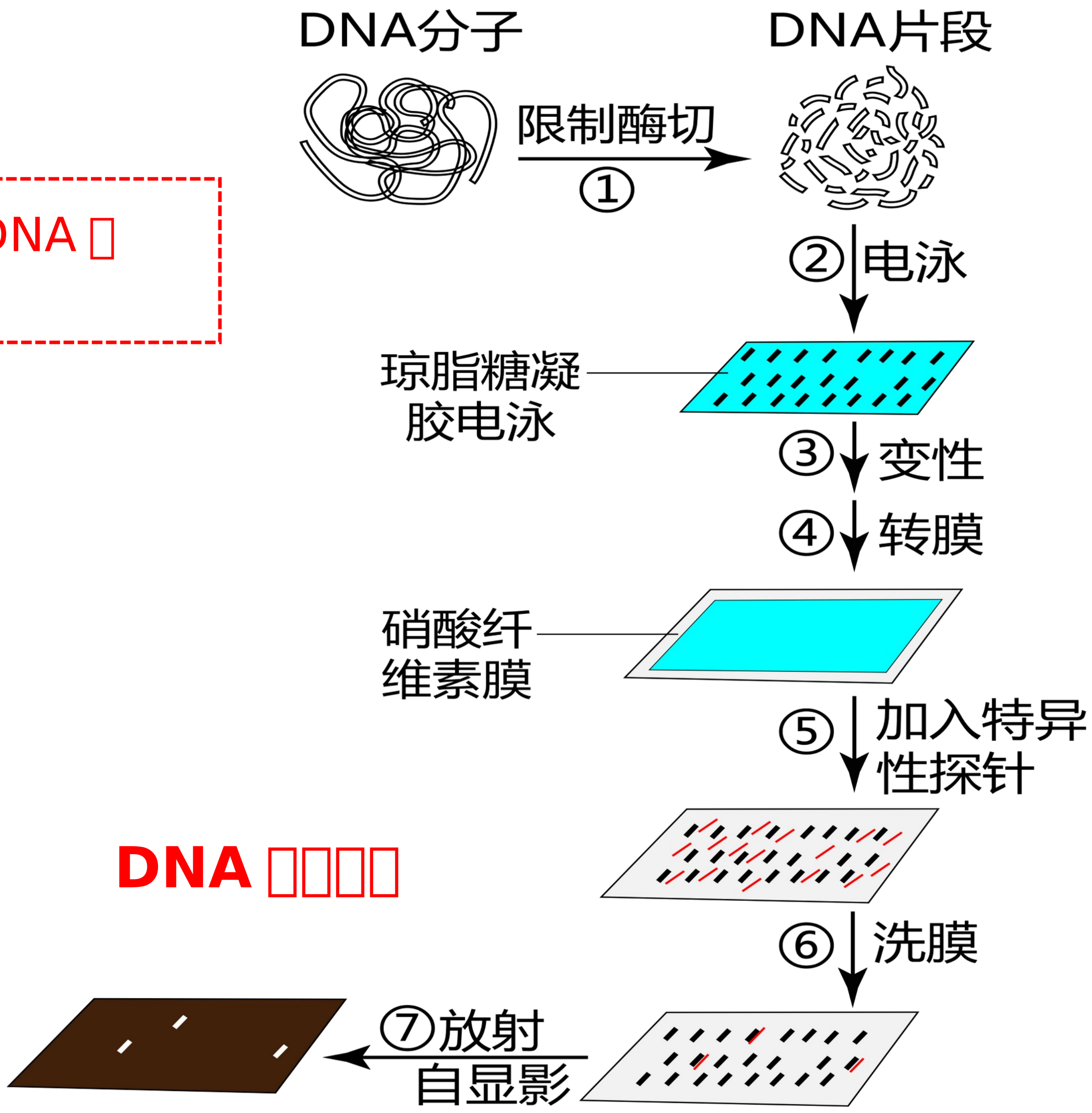
---- [illegible]

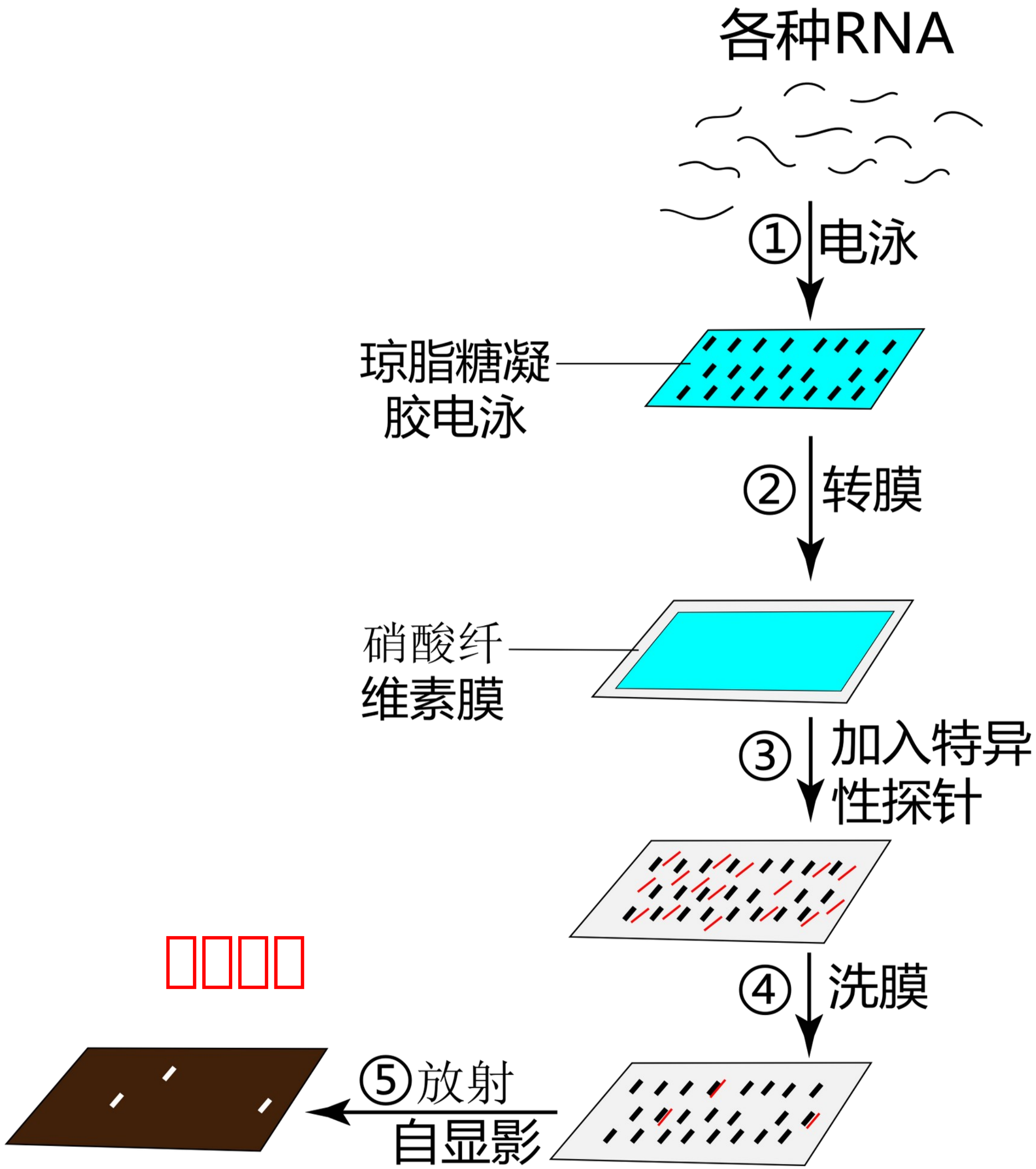
[illegible]

[illegible] DNA [illegible] RNA [illegible] DNA [illegible] RNA [illegible]

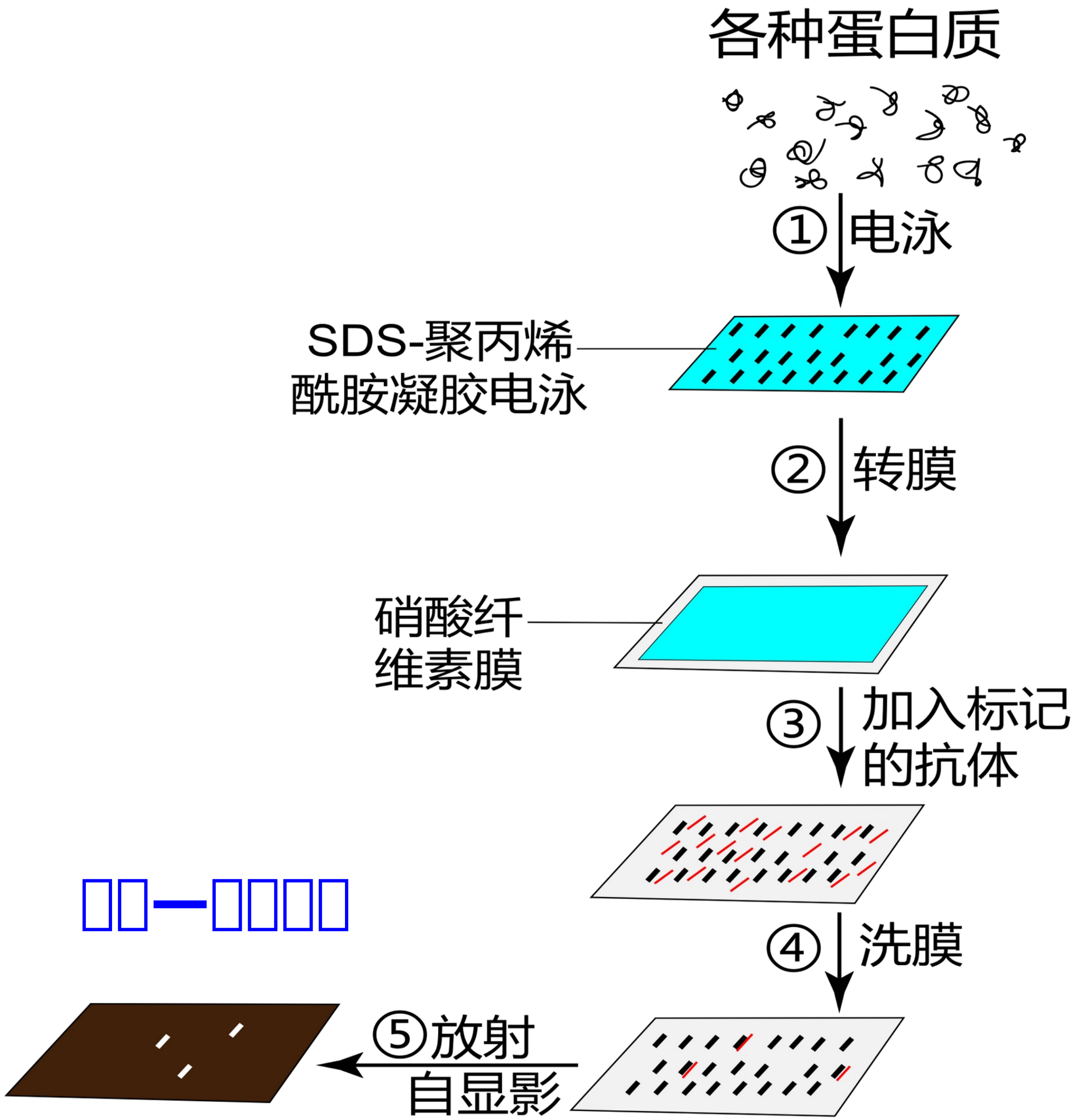


DNA [illegible]

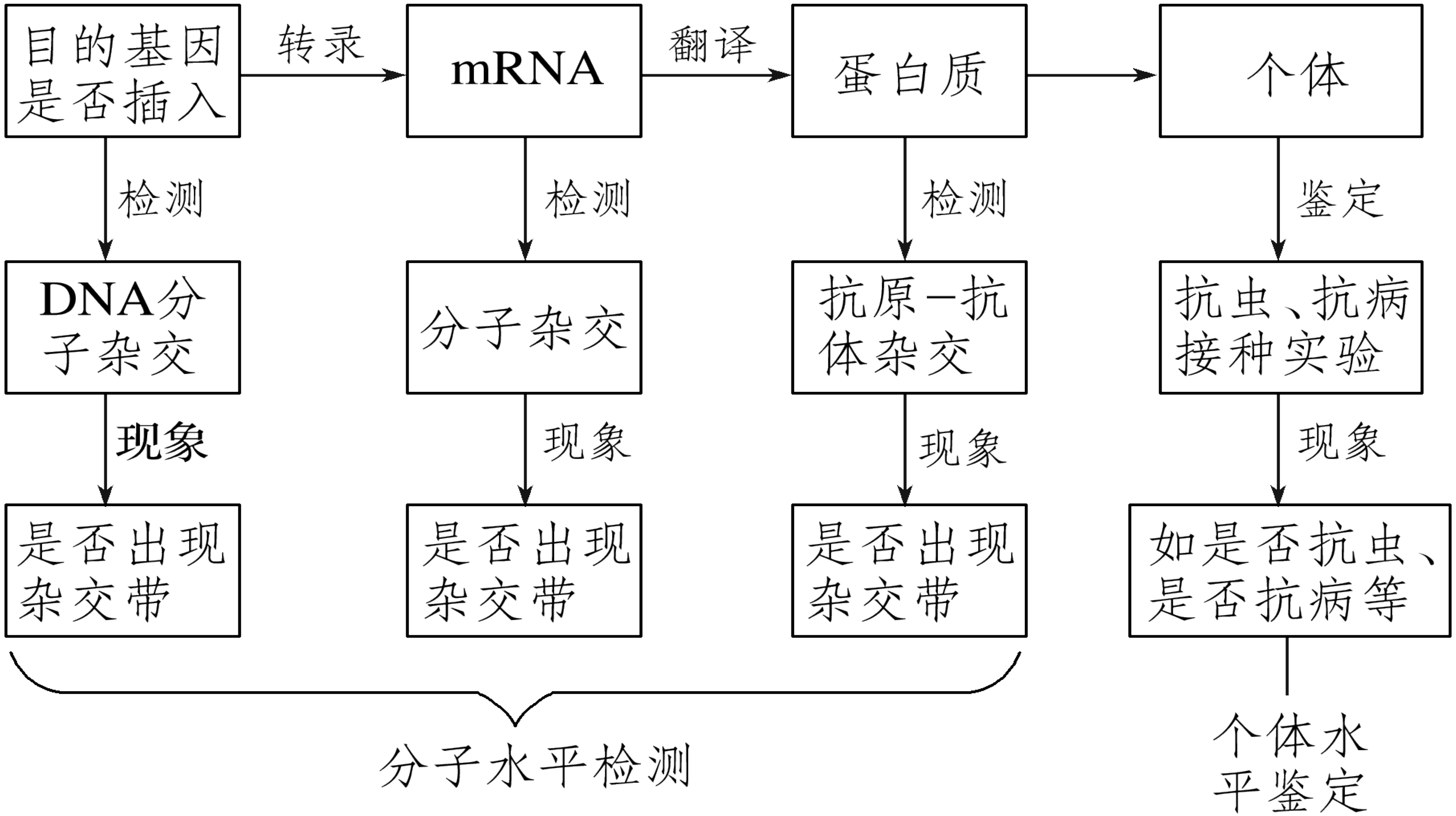


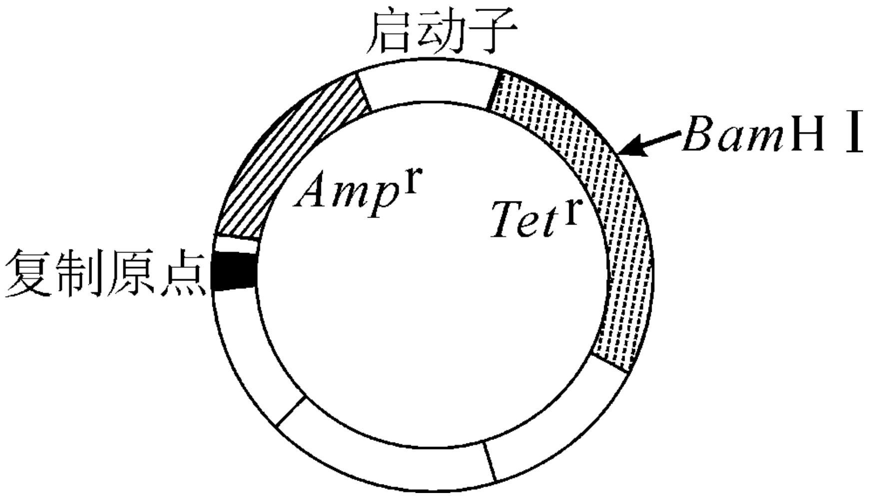


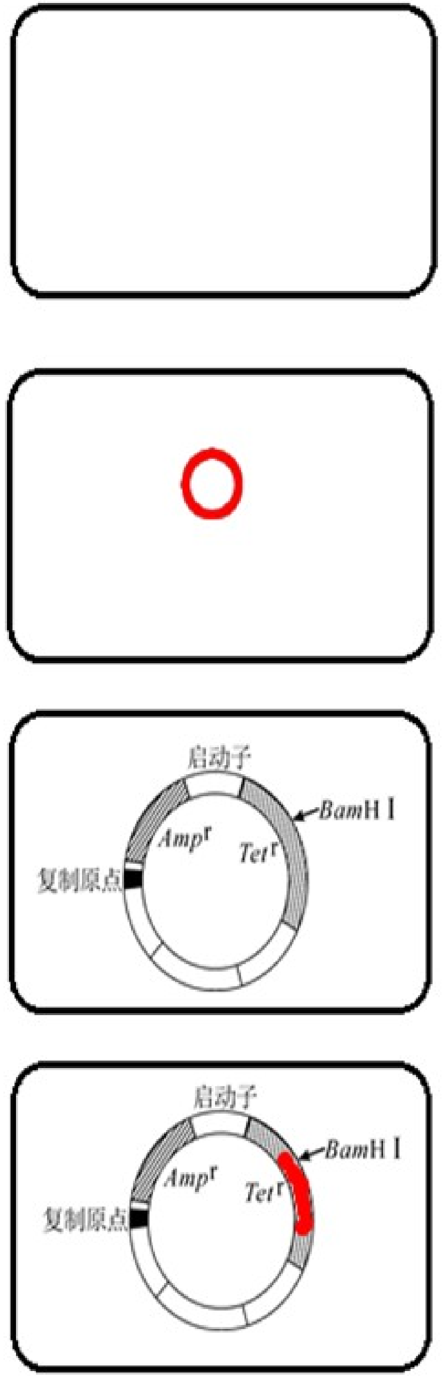
各种RNA
①电泳
琼脂糖凝胶电泳
②转膜
硝酸纤维素膜
③加入特异性探针
④洗膜
⑤放射自显影



各种蛋白质
①电泳
SDS-聚丙烯酰胺凝胶电泳
②转膜
硝酸纤维素膜
③加入标记的抗体
④洗膜
⑤放射自显影


目的基因是否插入
转录
mRNA
翻译
蛋白质
个体
检测
检测
检测
鉴定
DNA分子杂交
分子杂交
抗原-抗体杂交
抗虫、抗病接种实验
现象
现象
现象
现象
是否出现杂交带
是否出现杂交带
是否出现杂交带
如是否抗虫、是否抗病等
分子水平检测
个体水平鉴定





未被转化的大肠杆菌（普通大肠杆菌）

含有环状目的基因的大肠杆菌

含有质粒载体的大肠杆菌

含有插入了目的基因的重组质粒的大肠杆菌

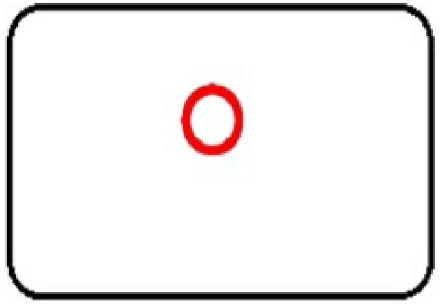
[illegible]2[illegible]

______________________________ [illegible]

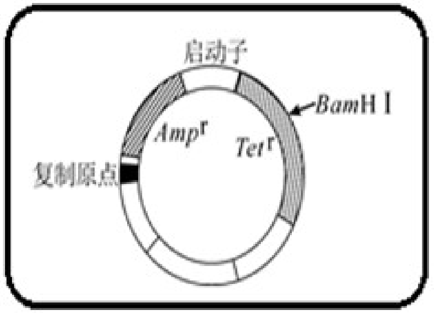
____

[illegible]

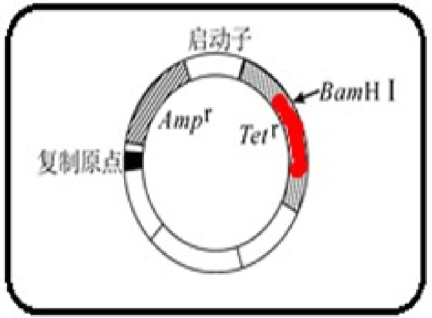
未被转化的大肠杆菌（普通大肠杆菌）



含有环状目的基因的大肠杆菌



含有质粒载体的大肠杆菌



含有插入了目的基因的重组质粒的大肠杆菌